

**ANTIOXIDANT-ESSENTIAL OIL GEL AS A TREATMENT FOR GINGIVITIS  
IN ORTHODONTIC PATIENTS**

A Thesis

by

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## **ABSTRACT**

The purpose of this study was to evaluate the treatment effect of an antioxidant-essential oil gel on orthodontic patients with generalized gingivitis. The gel contains the essential oils menthol and thymol, and the antioxidants ferulic acid and phloretin.

Thirty patients from the Texas A&M University Baylor College of Dentistry orthodontic clinic were screened for gingivitis, and randomly allocated into treatment and placebo-control groups. Each patient was evaluated at three orthodontic treatment visits (T1, T2, and T3). A periodontal examination, including probing depths (PD), bleeding on probing (BOP), gingival index (GI), and plaque index (PI) was performed at each visit. Between T1 and T2, patients were instructed to apply a topical gel (active or placebo) to their gingiva twice daily, after brushing. From T2 to T3 patients were instructed to discontinue use of the gel.

The treatment group showed statistically significant ( $p < .05$ ) reductions of BOP (-13.6 percentage points) and GI (-0.14) between T1 and T2, and significant increases in BOP (13.3 percentage points) and GI (0.14) between T2 and T3. Except for an increase in the GI between T2 and T3, the control group showed no significant changes in BOP or GI over time. The only other significant changes that occurred pertained to the treatment group, which showed significant increases in PD (0.08mm) and PI (0.18) between T2 and T3.

Application of a topical antioxidant-essential oil gel is an effective means of reducing inflammation in orthodontic patients with gingivitis.

## **DEDICATION**

This thesis is dedicated to my wife, Kerri, and to my parents Dennis and Gayle. Kerri, you have been a wonderful support through the last three years, and I know you will continue to be everything, and more, that I need and deserve in this life. Mom and Dad, you have provided me with all of the opportunities, love, and support that I could wish for. Thank you both so much for the loving example, and motivating influence that both of you have been in my life.

To my brother Adam: Thank you for being my mentor and for your encouragement, support, and advice as I have pursued my dream to become an orthodontist.

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## **CHAPTER I**

### **INTRODUCTION AND LITERATURE REVIEW**

Gingivitis is among the most common pathologies affecting the population, with a reported prevalence of over 50% and ranging up to 100%.<sup>1-3</sup> Gingivitis is common in all age groups, but some patterns are related to age. Around the time of puberty, the prevalence greatly increases, peaking between the ages of 9-14,<sup>3,4</sup> which should be of special interest to orthodontists considering the high number of adolescents in most orthodontic practices. From age 11-17 there is another trend to decrease in frequency, with a subsequent increase throughout the adult years; in the sixth decade, prevalence nears 100%.<sup>3</sup> Most commonly, gingivitis is associated with poor oral hygiene in both non-orthodontic and orthodontic patients. In conjunction with poor oral hygiene, increased mechanical plaque retention associated with fixed orthodontic appliances is one of the major reasons for high rates of gingivitis in orthodontic patients.<sup>5,6</sup> More recently, oxidative stress, and cytotoxic effects related to materials in fixed appliances and bonding agents have been implicated as factors causing gingival inflammation.<sup>7-10</sup>

Several modalities are available for treatment of gingivitis, including proper oral hygiene instructions, various dentifrices, and mouthwashes.<sup>11,12</sup> The current gold standard is use of chlorhexidine mouth rinse, with use of essential oil mouth rinses also providing effective therapy.<sup>13</sup> There is also a developing body of evidence to suggest that use of antioxidants may be helpful in the treatment of gingivitis.<sup>14-16</sup>

This review of the literature will first describe gingivitis and the biology of gingivitis. Then it will explore the relationship of orthodontics, gingivitis, and oxidative stress, including possible sequelae of gingivitis in orthodontic patients. Finally, a rationale for the use of antioxidants as a treatment modality for gingivitis in orthodontic patients will be presented.

### **Biological Basis of Gingivitis and Inflammation**

Bartold et al provide a concise review of the changes in gingival tissues during inflammation.<sup>17</sup> The underlying factor for all gingivitis is accumulation of plaque as the inciting agent. With this accumulation, an inflammatory response is initiated that involves numerous cell types, pathways, and molecular signals. The first line of defense is the junctional epithelium, which resides in the gingival sulcus; these cells respond to the insult with release of various cytokines, including interleukins 1 and 8 (IL-1 and IL-8), which are involved in neutrophil recruitment. Intercellular spaces in the junctional epithelium also begin to widen, allowing for movement of the inflammatory exudate into the gingival sulcus. At this point, if the inflammatory stimulus remains, neutrophil migration will continue along with increases in other immune cell populations, including polymorphonuclear lymphocytes (PMN) and T-lymphocytes. With prolonged inflammation, cytotoxic by-products of the inflammatory response result in apical migration of the junctional epithelium as the regeneration of new cells is insufficient to

out-pace the amount of tissue destruction; this denotes the earliest phase of pocket formation.

In addition to changes in the epithelium, the underlying connective tissue is also heavily involved in the inflammatory response. The major immune cell components within the connective tissue are PMNs, T-lymphocytes, and neutrophils. Platelet derived growth factor (PDGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), prostaglandin-E<sub>2</sub> (PGE<sub>2</sub>), interferon- $\gamma$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1, IL-6 and IL-8 are some of the major signaling molecules released from inflammatory cells that affect behavior of fibroblasts in the connective tissue matrix. These molecules lead to varied responses, which include induction of mitosis (PDGF), increased collagen and proteoglycan production (TGF- $\beta$ ), increase in collagenase production (TNF- $\alpha$ , IL-1), inhibition of collagen synthesis (PGE-<sub>2</sub>, interferon- $\gamma$ , TNF- $\alpha$ ), and other processes leading to matrix degradation (IL-1, IL-6, IL-6, IL-8, interferon- $\gamma$ ). Interferon- $\gamma$ , IL-1 and IL-6 also stimulate cell-to-cell adhesion between fibroblasts and neutrophils. These interactions are shown to increase cytotoxicity through the generation of oxygen-derived free radicals.

In summary, the junctional epithelium of the gingival sulcus is the first responder when confronted with bacterial challenge. This can lead to involvement of the underlying connective tissue and a prolonged inflammatory response, with destruction of tissue, if the inciting agent is not removed.

## **Increased Inflammation due to Orthodontic Appliances**

Orthodontic appliances have been consistently linked to increased inflammation of the gingiva. Reasons cited include mechanical plaque retention, changes in the composition of oral microflora following placement of orthodontic appliances, and oxidative cytotoxic byproducts originating from orthodontic materials.

Zachrisson and Zachrisson evaluated the condition of the gingiva during orthodontic treatment.<sup>5</sup> Their study included 49 patients treated with edgewise appliances, and a control group of 53 subjects matched for age and sex from the local schools. Measurements were taken at the buccal and mesial surfaces of the upper right first molars, second premolars, canines, and central incisors. Patients in the treatment group typically exhibited generalized moderate gingivitis within one to two months after appliance placement. This change was noted despite low plaque indices, which was due to intensive oral hygiene instruction. Gingival index scores in the treatment group (interproximal,  $1.78 \pm 0.28$ ; buccal,  $1.33 \pm 0.37$ ) were higher than those of the control group (interproximal,  $1.23 \pm 0.36$ ; buccal,  $0.92 \pm 0.49$ ) even though the plaque indices of the treatment group were lower than scores in the control group ( $0.49 \pm 0.41$  vs.  $0.68 \pm 0.61$ ).

Kloehn and Pfeifer also evaluated the periodontal condition of orthodontic patients, examining 50 consecutive cases before, during, and following orthodontic treatment.<sup>18</sup> Prior to treatment, a periodontal exam was performed. This exam was repeated every 3 months during treatment, 48 hours after removal of appliances, and

again four months later. Patients were also provided with oral hygiene instructions throughout the course of treatment. Using Russell's periodontal index (lower scores indicate periodontal health), the number of patients scoring "0" decreased from 20% to 10% after eight months of treatment.<sup>19</sup> Patients were also noted to have an increase in gingival hyperplasia during treatment, especially in the premolar and molar regions. Evaluation of oral debris using Greene and Vermillion's method showed a slight decrease in plaque after initiation of treatment.<sup>20</sup> Following removal of appliances, the subjects' periodontal condition improved, and no detrimental effects were noted as a result of orthodontic treatment. The authors attributed the increased inflammation during treatment to the presence of orthodontic materials in the mouth, including bands, cements, and the proximity of the wires to the tissue in the posterior regions.

In another study looking strictly at the second molars, gingivitis developed within one month of placement of either a bonded tube or a cemented band.<sup>6</sup> While the level of gingival inflammation was higher with cemented bands than with bonded tubes, both groups showed higher gingival index scores following placement of the orthodontic appliances than untreated controls. They also showed elevated plaque index scores. The authors cited increased mechanical plaque retention of the orthodontic attachments and their impingement on the proximity of gingival tissues as possible reasons for the findings.

Sixty patients were enrolled in a study to evaluate the changes that occur in the subgingival microbiota during orthodontic treatment.<sup>21</sup> Experimental subjects were recruited from patients who were to receive orthodontic treatment; control group

subjects did not receive orthodontic treatment (n=30 for both groups). Patients in the experimental group were evaluated prior to appliance placement and 3 months following appliance placement; subjects in the control group were evaluated only once. The results corroborated previous findings of increased bleeding on probing (baseline experimental group,  $11.6 \pm 14.6\%$ ; 3 month experimental group,  $19.4 \pm 21.9\%$ ; control group,  $4.4 \pm 8.6\%$ ), higher gingival index (baseline experimental group,  $0.2 \pm 0.3$ ; 3 month experimental group,  $0.4 \pm 0.5$ ; CG,  $0.1 \pm 0.2$ ), and a higher plaque index (baseline experimental group,  $7.0 \pm 5.0\%$ ; 3 month experimental group,  $9.2 \pm 5.0\%$ ; CG,  $5.1 \pm 2.6\%$ ) following appliance placement when compared to baseline initial presentation and compared to the control group. Additionally, they showed a change in the make-up of the plaque bacteria, with elevated levels of *Porphyromonas gingivalis*, *Prevotella intermedia/Prevotella nigrescens*, *Tannerella forsythia* and *Fusobacterium* species. The experimental group also demonstrated the presence of superinfecting bacteria that can lead to periodontitis.

A similar study evaluated patients 1 week, 3 months, and 6 months following placement of fixed appliances. The pathogenic bacteria *Tannerella forsythia* and *Prevotella nigrescens* were significantly increased, while others were increased but did not show statistically significant differences between the treated and control groups.<sup>22</sup>

## **Oxidative Stress and Inflammation in Periodontal Tissues**

Reactive oxygen species (ROS) are involved in tissue damage through a variety of different mechanisms, including DNA damage, lipid peroxidation, protein damage, oxidation of enzymes, and stimulation of pro-inflammatory cytokine release.<sup>23</sup>

ROS are found in all living cells as a result of metabolism, and can also be introduced to the cells via interaction with compounds surrounding or near the cells. As oxidative stress increases, and if self-contained antioxidant mechanisms are unable to neutralize the build-up of ROS, cellular damage can result.<sup>9</sup>

Wei et al established links between increased oxidative stress and periodontal disease.<sup>7</sup> Nineteen patients with periodontal disease were compared to 8 control subjects that were free of periodontitis. Gingival crevicular fluid from both groups was examined for several markers of oxidative stress, including glutathione peroxidase, lactoferrin, myeloperoxidase and interleukin-1 $\beta$ . The disease group demonstrated higher levels of each of these substances, and their levels were positively correlated with the plaque index, gingival index, probing depth, and probing attachment level. The authors concluded that the levels of these substances could result in damage from reactive oxygen species.

A similar study by Patel et al confirmed the higher level of oxidative insult present in periodontal disease. Sixty subjects were recruited into 3 groups; healthy, gingivitis, and periodontitis.<sup>10</sup> Gingival crevicular fluid from each group was analyzed for plasma glutathione peroxidase, a marker for oxidative stress. The levels of plasma

glutathione peroxidase were positively correlated with the severity of periodontal disease, with the healthy group showing the lowest levels, and the periodontitis group showing the highest levels.

Associations have also been found between decreased levels of salivary antioxidant capacity and periodontal disease. Sculley and Langley-Evans evaluated 129 patients during a routine dental check-up; in addition to analyzing each patient's salivary antioxidant capacity, they characterized each patient's periodontal condition based on the Community Periodontal Index of Treatment Needs system.<sup>24</sup> Patient's with lower antioxidant capacity, specifically lower levels of urate, scored lower on the index. The authors concluded that there is an association between reduced salivary antioxidant capacity and increased oxidative damage in the periodontium.

### **Sequelae of Gingivitis in Orthodontics**

With the increased risk and severity of gingivitis in orthodontic patients, it is important to understand the possible sequelae that go along with this, and what can occur with prolonged inflammation.

Gingivitis by itself poses certain risks to periodontal health, which can become amplified by fixed appliances. While it has been suggested that the inflammatory changes, including increased gingival index and pocket depths, are largely transient during orthodontic treatment,<sup>5,6</sup> the fact remains that prolonged inflammation can have permanent detrimental effects on the periodontium.



If the inflammation has progressed beyond gingivitis to periodontitis with bony involvement, the implications can be quite serious in regards to attachment loss. If the subgingival plaque and calculus are not controlled, any tooth movement may accelerate loss of attachment.<sup>25</sup> Animal studies have shown that tooth movement into an infrabony pocket will result in additional bone loss compared to control teeth with a pocket and no tooth movement.<sup>26, 27</sup>

Another issue that may arise with gingivitis during orthodontic therapy is gingival hyperplasia. Consistently poor oral hygiene may lead to gingival hyperplasia, especially in the lower incisor region. This can be difficult to differentiate from superficial fibrosis, and should be investigated to evaluate if there is any evidence of attachment loss, which can occur in severe cases of gingival hyperplasia.<sup>25, 28</sup> Much of the risk from gingival hyperplasia comes from the environment that is created by increasing pocket depth, and in susceptible patients may lead to periodontitis. Palomo et al also argued that it is impossible to differentiate between a relatively innocuous pseudopocket and gingival hyperplasia that will lead to actual attachment loss.<sup>28</sup>

Zachrisson and Alnaes evaluated a group of 51 patients for attachment loss at four sites (first molar, second premolar, canine, central incisor) during orthodontic treatment.<sup>29</sup> When compared to non-treated controls, the patients exhibited small (0.30mm mean), but significant attachment loss at 3 of the 4 selected sites (second premolar, canine, central incisor). While they were unable to specifically determine what the cause of the attachment loss was, they emphasized the potential role of gingivitis because of its influence on the periodontium. They also cited examples from

an earlier study by Rateitschak et al,<sup>30</sup> which showed some orthodontic cases with evident gingivitis and the beginning of apical migration of the pocket epithelium.

It is evident that the inflammation occurring with gingivitis is conducive to periodontal breakdown if left untreated. Additionally, the inflamed oral environment that accompanies gingivitis makes it more difficult to keep the enamel clean and healthy. This can lead to white spot lesions and caries. Tanner et al evaluated a sample from the Boston Children's Hospital dental department.<sup>31</sup> Sixty subjects were recruited sequentially; 30 with white spot lesions, and 30 without. They described a strong correlation between gingivitis and the detection of enamel white spot lesions in their sample. Patients with white spot lesions showed a higher mean gingival index, but did not differ in plaque index, compared to those without lesions.

### **Cytotoxicity, Oxidation, and Inflammation Associated With Orthodontic Materials**

Many of the materials commonly used in orthodontics are known to increase oxidative stress and are linked to other undesirable processes. Iron, copper, chromium, and vanadium undergo a redox cycle that generates free radicals<sup>32</sup>, and alumina ceramics have shown increased chromosomal anomalies and weak genotoxicity.<sup>33</sup> Several studies have confirmed these types of findings with specific use of orthodontic products in-vitro.

Spalj et al evaluated several different types of archwires to determine the amount of oxidative stress induced by each.<sup>9</sup> In their in-vitro study, mouse fibroblast cells were

exposed to the different types of archwires. After 48 hours the viability of the cells was recorded and the cells' DNA were analyzed for oxidative damage. The results showed no acute toxicity to the cells, but there was a statistically significant difference between some metals. Nickel-titanium resulted in the lowest cell viability, and was significantly lower than the negative and absolute control, and all archwires except titanium-molybdenum. Nickel-titanium also showed the highest level of oxidative stress; higher than all wires and controls ( $P<0.05$ ).

Buljan et al exposed different types of brackets to simulated intraoral conditions to determine levels of cell viability and increased oxidative stress.<sup>34</sup> Seven different types of brackets (stainless steel, monocrystalline sapphire ceramic, aluminum oxide/cobalt-chrome, aluminum oxide/nickel-cobalt and rhodium, polyurethane, stainless steel/nickel-titanium, and polycarbonate stainless steel) were placed in artificial saliva and stored for 30 days at 37°C. Additionally, a positive control ( $H_2O_2$ ) and negative control (artificial saliva only) were stored under the same conditions. Following this phase, the artificial saliva that had been exposed to the various brackets was placed on murine fibroblast cell cultures, and the effects on cell viability and oxidative damage were evaluated.

The positive controls exhibited the lowest cell viability ( $89.93\pm3.07\%$ ), and was significantly different from all brackets except the polyurethane ( $94.93\pm3.03\%$ ). Monocrystalline sapphire ceramic showed the greatest amount of cell number increase, but was not significantly greater than that seen in other brackets or the positive control group. The highest levels of oxidative stress were noted for the stainless steel

( $1.44 \pm 0.36$  8-OHdG, ng/ml) and stainless steel/nickel-titanium brackets ( $1.42 \pm 0.15$ ). However, all brackets with the exception of the monocrystalline sapphire ceramic ( $1.09 \pm 0.42$ ), demonstrated higher oxidative stress than the positive control ( $1.10 \pm 0.50$ ).

The authors concluded that all brackets, regardless of the material properties, are a source of in-vitro oxidative stress, with stainless steel and polyurethane being the least biocompatible. Conventional ceramic brackets showed the highest level of biocompatibility.

An earlier study by Grimmsdottir et al investigated the cytotoxic potential of orthodontic materials by means of an agar overlay test with mouth fibroblast cells.<sup>8</sup> Materials were evaluated based on the presence or absence of zones of lysis surrounding the orthodontic material, and on the extent of the zones. None of the archwires induced cell lysis, despite high levels of cytotoxic nickel. However, devices containing high levels of brazing material, such as molar bands, face bows, and some brackets, showed high levels of cytotoxicity, with large areas of cell lysis surrounding them.

### **Essential Oils and Treatment of Gingivitis**

The use of essential oils is well documented in the literature, and they have been proven effective in reducing gingivitis in orthodontic patients as well as the general population.<sup>35, 36</sup> Gunsolley et al carried out a systematic review which concluded that use of essential oil mouth rinses provides improved oral hygiene and reduced plaque and

gingivitis. Additionally, Tufekci et al showed that use of Listerine, an essential oil mouth rinse, reduces the amount of plaque and gingivitis in orthodontic patients.<sup>35</sup>

### **Antioxidants in Prevention of Plaque and Inflammation**

With the links between oxidative stress and inflammation becoming more clearly defined, promising research is being conducted on the use of antioxidants to control inflammation. Sendamangalan et al looked specifically at several polyphenolic compounds, including gallic acid, tannic acid, quercetin and salicylic acid.<sup>14</sup> Each of these compounds was tested for antimicrobial capability against *Streptococcus mutans* and also for their antioxidant capacity. Of these compounds, tannic acid showed the highest antimicrobial activity, and also a high antioxidant capability. However, the high antimicrobial capability of tannic acid results in cytotoxicity if the concentration is too high, making it difficult to obtain a high antioxidant capacity at safe levels intraorally. Salicylic acid showed the lowest level of antimicrobial activity and the weakest antioxidant capacity. Gallic acid and quercetin showed high levels of antioxidant capacity in addition to good antimicrobial capability, making them better candidates for use intraorally.

The same authors evaluated the effects of these polyphenolic compounds against specific enzymes involved in the biofilm formation of dental plaque: glucosyl and fructosyl transferase.<sup>15</sup> These enzymes aid in the conversion of sucrose to glucose and fructose, and also in the process of polymerizing glucose and fructose to glucans and

fructans. These glucans and fructans chains are integral to the adhesion of plaque bacteria to the tooth structure. Ascorbic acid (the positive control) and quercetin showed a 45% inhibition of these enzymes compared to a negative control. Quercetin also showed a 75% reduction in biofilm formation compared to 76% reduction for ascorbic acid. Gallic acid only showed a 15% reduction in enzyme capacity, but an 82% reduction in biofilm formation, suggesting that the biofilm inhibition is accomplished by means other than enzyme inhibition. Tannic acid reduced enzyme activity by 32% and biofilm formation by 66%. Salicylic acid showed the lowest inhibition of enzyme activity and the lowest reduction in biofilm formation.

Other research on polyphenols has been performed using substances extracted from the *Lonicera caerulea* fruit (sweetberry honeysuckle).<sup>16</sup> Human gingival fibroblasts were harvested from healthy donors during third molar extractions. Cells were cultured, then exposed to *E. coli* lipopolysaccharides (LPS) to induce reactive oxygen species/oxidative damage and inflammation. Polyphenolic fractions of *L. caerulea* (PFLC) were also added to the samples to evaluate its antioxidant capability. Intracellular glutathione (GSH, an antioxidant already present in cells) was measured for its level of depletion. Levels of pro-inflammatory cytokines and enzymes such as IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , iNOS, and COX-2 were measured to gauge the inflammatory response of the cells. The amount of lipid peroxidation was also measured to directly evaluate antioxidant capacity. Results of the study showed no harmful effects from the PFLC; no visible effects of cytotoxicity were observed on the control samples exposed to the PFLC. In the absence of LPS, PFLC had no effect on GHS levels. In LPS exposed

cells, the levels of GSH were markedly decreased. However, when treated with PFLC, cells that had been exposed to LPS showed less depletion of GSH, suggesting good antioxidant capacity of the PFLC. When directly measured, the amount of lipid peroxidation was greatly decreased by applying PFLC to LPS exposed cells. Levels of oxidation in PFLC treated cells approached the level seen in cells that were not exposed to LPS. In addition to antioxidant capacity, effects were also seen in the evaluation of the inflammatory mediators. Application of PFLC alone decreased IL-6, but had no effect on the level of IL-1 $\beta$  or TNF- $\alpha$ . Exposure to LPS generated increases in IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 compared to untreated cells. In LPS-exposed cells, treatment with PFLC at the highest level (50  $\mu\text{g ml}^{-1}$ ) showed marked reductions in IL-1 $\beta$ , TNF- $\alpha$ , and IL-6. Western blot analysis showed that PFLC treated cells also showed a reduction in COX-2 expression (greatest effect at 25  $\mu\text{g ml}^{-1}$ ), but had no effect on iNOS expression at any concentration.

Ferulic acid and phloretin are two other phenolic antioxidant compounds that have been shown to have anti-inflammatory capabilities.<sup>37, 38</sup> Phloretin is one of several polyphenolic compounds found in apples, and ferulic acid is available in many common food items, including wheat, oranges, tomatoes, carrots, and sweet corn. Jung et al showed reduced expression of NF- $\kappa$ B, and other pro-inflammatory genes in various cultured cancer cell lines when treated with phloretin.<sup>38</sup> Another in-vitro study using splenocytes showed a reduction in the levels of inflammatory mediators such as prostaglandin E2 and tumor necrosis factor-alpha with the introduction of ferulic acid and its metabolites.<sup>39</sup>

There is also research regarding the use of monounsaturated fatty acids (MUFA) in the treatment of periodontal disease. MUFAs have been shown to decrease low-density-lipoprotein oxidation,<sup>40</sup> which partially led to the investigation of these compounds in combatting periodontal disease. There is recent evidence to support the acceleration of periodontal disease by lipid peroxidation, caused specifically by *Aggregatibacter actinomycetemcomitans*.<sup>41</sup> Hasturk et al evaluated the effects of one such MUFA, 1-tetradecanol complex (1-TDC) in experimentally induced periodontitis in rabbits.<sup>42</sup> Fifteen animals were randomly assigned to treatment and control groups after the induction of experimental periodontitis with ligature wires and *Porphyromonas gingivalis*. After 6 weeks, measurements were taken and labeled as baseline periodontitis. Probing depth at baseline was  $3.9 \pm 1.1$ mm. The treatment group received 1-TDC as a topical application to the diseased sites, the placebo group received mineral oil, and the control group was left untreated. After another 6 weeks, periodontal measurements were taken again. The untreated group and placebo group showed similar results with continued progression of disease (probing depth at placebo sites was  $6.2 \pm 0.3$ mm). The 1-TDC treated rabbits demonstrated decreased probing depths ( $3.2 \pm 0.6$ mm), showing that the progression of periodontitis had been stopped and inflammation was decreased. Radiographic analysis corroborated the macroscopic findings and showed that the bone loss was stopped, and actually showed some regeneration of the bone. Sites that were treated with placebo or left untreated showed an additional 3% and 5% bone loss, respectively, compared to baseline periodontitis.



## **Clinical Use of Antioxidants in Treatment of Periodontal Disease**

The use of antioxidants as a treatment modality for periodontal disease is a developing field. However, recent clinical trials have shown promising results in their ability to reduce inflammation.

Chapple et al evaluated the effect of an antioxidant and phytonutrient rich fruit, vegetable and berry juice powder versus placebo.<sup>43</sup> Sixty volunteers with chronic periodontitis and nutritionally replete blood chemistry were enrolled into three groups: fruit/vegetable juice powder (FV), fruit/vegetable/berry juice powder (FVB), and placebo. Subjects received non-surgical periodontal therapy, and were asked to take daily supplements in addition to their clinical treatment. Outcome variables were evaluated at 2, 5, and 8 months following completion of treatment.

Outcomes improved in all groups at two months, with an additional reduction in probing depth of 0.22 mm noted in the FV group versus placebo ( $P < 0.03$ ). Percent bleeding on probing was also lower at five months in the FV group versus placebo ( $P < 0.05$ ). The authors concluded that there are initial improvements in probing depth and bleeding on probing in patients taking the antioxidant and phytonutrient supplements. However, these were not sustained at the 8 month follow up.

Chandra et al evaluated another systemic antioxidant composition in patients with gingivitis.<sup>44</sup> The antioxidant capsules contained primarily lycopene in addition to vitamin A, vitamin C,  $\beta$ -tocopherol acetate, and other antioxidants. Twenty patients were randomized into two groups, one receiving the antioxidant capsules, and the other a

placebo. Across both groups the patients were further randomized into a split mouth design, with each patient receiving two quadrants of oral prophylaxis (OP) immediately after baseline measurements were taken. The other two quadrants remained untreated (non-OP). Outcome measures were taken at one and two weeks, and included subgingival bleeding index (SBI), plaque index (PI), and gingival index (GI).

Results showed that all groups demonstrated significant reductions in GI relative to baseline at one and two weeks. The OP-lycopene group showed the greatest percentage reduction in GI ( $30.02 \pm 7.17\%$ ), which was statistically significant ( $p < 0.05$ ) compared to the non-OP placebo ( $11.94 \pm 6.55\%$ ) and the OP-placebo group ( $18.85 \pm 7.29\%$ ). The non-OP-lycopene, with a  $24.14 \pm 4.81\%$  reduction, was significantly different from the non-OP-placebo group.

The SBI showed similar results, with all groups showing significant reductions from the baseline at one and two weeks. The OP-lycopene ( $38.42 \pm 9.81\%$ ) and OP-placebo ( $36.94 \pm 11.61\%$ ) groups showed the greatest percentage reductions in SBI. Additionally the OP-lycopene group showed a significant difference ( $p < 0.05$ ) in comparison with the non-OP-placebo group ( $23.96 \pm 12.4\%$ ).

Based on these results the authors concluded that there may be an additive effect using routine oral prophylaxis along with antioxidant treatment.

## **Purpose**

Based on this review of the literature, there is an elevated risk of gingivitis and oxidative stress in orthodontic patients. At this point there are a limited number of clinical trials evaluating the use of antioxidants in the treatment of gingivitis, and none include orthodontic patients. There are also no clinical trials evaluating the use of ferulic acid or phloretin in the treatment of gingivitis.

This study is a randomized, double-blinded placebo-controlled clinical trial. Our aim is to evaluate the efficacy of a topical gel containing ferulic acid and phloretin, in addition to essential oils, in the treatment of gingivitis in orthodontic patients (AO ProVantage Dental Gel, Periosciences, Dallas, TX).

## **CHAPTER II**

### **BACKGROUND**

Gingivitis is among the most common pathologies affecting the population, with a reported prevalence of over 50%.<sup>1-3</sup> While gingivitis is common in all age groups, prevalence increases with age. Around the time of puberty, the prevalence greatly increases, peaking between the ages of 9-14,<sup>3,4</sup> which is important for orthodontists considering the high number of adolescents they treat. Between the ages of 11-17, there is a tendency for the prevalence of gingivitis to decrease, followed by an increase throughout the adult years; by the sixth decade, prevalence approaches 100%.<sup>3</sup> Gingivitis is associated with poor oral hygiene, and increased mechanical plaque retention associated with fixed orthodontic appliances is one of the major reasons for higher rates of gingivitis among orthodontic patients.<sup>5,6</sup> More recently, oxidative stress, and cytotoxic effects of materials in fixed appliances and bonding agents have been implicated as factors causing gingival inflammation.<sup>7-10</sup>

Several modalities are available for treating gingivitis, including proper oral hygiene instructions, various dentifrices, and mouthwashes.<sup>11,12</sup> While essential oil mouth rinses provide effective therapy, the current gold standard is use of chlorhexidine mouth rinse.<sup>13</sup> There is also a developing body of evidence to suggest that antioxidants may be useful in the treatment of gingivitis.<sup>14-16</sup>

However, there are a limited number of clinical trials evaluating the use of antioxidants in the treatment of gingivitis, and none include orthodontic patients. These

trials have found decreased severity of gingivitis, decreased bleeding on probing, and modest reduction in pocket depth.<sup>43, 44</sup> There are also no clinical trials evaluating the use of ferulic acid or phloretin in the treatment of gingivitis. As such, the aim of the present study is to evaluate the efficacy of a topical gel containing ferulic acid and phloretin, in addition to essential oils, in the treatment of gingivitis in orthodontic patients.

### **CHAPTER III**

#### **MATERIALS AND METHODS**

The study pertains to 32 patients who were undergoing comprehensive treatment at Texas A&M University Baylor College of Dentistry. Eligible patients had to be 12-65 years old, and have bonded brackets in both arches from first premolar to first premolar, or from second premolar to second premolar when the first premolars had been extracted. Patients also had to exhibit a minimum of 30% bleeding on probing at qualifying sites, including all bonded teeth mesial to the first molars, and not adjacent to a fully banded tooth.

Exclusion criteria included patients with syndromes, patients with systemic diseases that may contribute to inflammatory processes (such as lichen planus, systemic lupus erythematosus, and benign mucus membrane pemphigoid disease), pregnancy, active caries, and periodontally compromised teeth. Before enrollment, informed consent to participate was obtained from each patient; the study was approved by the Institutional Review Board at the Texas A&M University Baylor College of Dentistry.

The study was double-blinded and placebo controlled. Upon enrollment, patients were randomly assigned to either a placebo-controlled group or an active treatment group based on a pre-determined randomly generated list. The treatment group contained 7 males ( $16.1 \pm 1.1$  years of age) and 8 females ( $15.9 \pm 2.2$  years of age), and the placebo group contained 7 males ( $16.8 \pm 2.1$  of age) and 8 females ( $15.1 \pm 1.9$  years of age). The active treatment group received a gel containing the antioxidants phloretin

and ferulic acid, in addition to essential oils (AO ProVantage Dental Gel, Periosciences, Dallas, TX). The placebo gel contained neither the antioxidants nor essential oils.

All of the patients were instructed, and shown how to apply a pea-sized amount of gel to their gingiva twice a day, immediately after brushing. The gel was applied to their buccal and lingual/palatal gingiva. They were then instructed to thoroughly expectorate after 30 seconds, and to avoid rinsing, eating or drinking for 30 minutes. Patients were instructed to continue this regimen twice a day until their next regularly scheduled orthodontic treatment visit (most approximately 4 to 6 weeks).

At the initial visit (T1), each patient received a periodontal examination, which included probing depths (PD), bleeding on probing (BOP), plaque index<sup>45</sup> (PI), and gingival index<sup>45</sup> (GI). Each patient also received oral hygiene instructions; tooth brushing was demonstrated using the bass technique,<sup>46</sup> which was modified to clean both gingival and occlusal to the brackets. Flossing was demonstrated using floss threaders (Gum Eez-Thru, Sunstar Americas Inc, Chicago, IL).

At the patients' next regularly scheduled visit (T2), they received another periodontal examination, and were instructed to discontinue use of the gel, but to continue with a proper oral hygiene regimen until their next visit. At the follow-up visit (T3), each patient again received a periodontal examination (Figure 1).

The interval from T1 to T2 in the treatment and control groups were  $35.7 \pm 10.8$  days and  $42.6 \pm 23.5$  days, respectively. The entire study period, from T1 to T3 was  $78.3 \pm 29.5$  days for the treatment group, and  $92.8 \pm 33.9$  days for the control group. The variability in duration was due to differences in orthodontic treatment intervals and

missed appointments. There were no statistically significant group differences in duration between T1 and T2 ( $p=0.775$ ) or between T2 and T3 ( $p=0.239$ ). Based on the experimental gingivitis model, these intervals are judged to be sufficient for both the resolution, and development of gingivitis.<sup>47</sup>

## **Analysis**

Each periodontal exam was performed by a single investigator using a UNC periodontal probe and #5 explorer (Hu-Friedy, Chicago, IL). PD was recorded at six sites (distobuccal, facial, mesiobuccal, distolingual, lingual, mesiolingual) on each tooth. After waiting for 30 seconds, BOP was assessed visually at the same sites and recorded as present or absent. GI and PI were recorded using the Silness-Löe plaque and gingival indices.<sup>45</sup> Probing depth, gingival index, and plaque index were all expressed as averages for each tooth, while bleeding on probing was expressed as a percentage of sites for each tooth.

## **Statistics**

SPSS version 22 (SPSS Inc.; Chicago, IL) was used to analyze the data, using  $p<0.05$  significance level. Mean and standard deviation were utilized as descriptive statistics because the data were normally distributed. However, due to small sample size, the Mann-Whitney U test was used to evaluate differences between the control and



treatment groups, and the Wilcoxon signed ranks test was used to evaluate differences between time points within the groups.

## **CHAPTER IV**

### **RESULTS**

During the study, one female from the treatment group and one male from the control group dropped out. On the day they were scheduled for their T2 evaluation, both of them stated that they no longer wished to continue in the study due to the discomfort associated with the periodontal probing. The data from their T1 evaluations were not included in the results.

#### **Bleeding on Probing**

At the initial T1 examination, BOP occurred at  $62.9 \pm 12.9\%$  and  $72.1 \pm 10.7\%$  of the sites in the treatment and placebo groups, respectively. The group difference was statistically significant ( $p=0.046$ ) (Figure 2) (Table 1). From T1 to T2, the treatment group showed a statistically significant ( $p=0.002$ )  $13.6 \pm 10.2$  percentage point reduction in BOP compared to a statistically insignificant ( $p=0.691$ )  $3.0 \pm 12.5$  percentage point reduction in the placebo group, which was a significant ( $p=0.007$ ) group difference (Figure 3) (Table 2). This produced a statistically significant ( $p=0.002$ ) group difference at T2, with BOP at  $49.2 \pm 15.7\%$  and  $69.1 \pm 16.7\%$  of the sites in the treatment and placebo groups, respectively. The treatment group also showed a statistically significant ( $p=0.016$ ) increase of  $13.3 \pm 4.4$  percentage points in BOP between T2 and T3, while the  $7.6 \pm 15.4$  percentage point increase in the control group was not statistically

significant ( $p=0.124$ ). At T3,  $61.2 \pm 12.8\%$  of the sites in the treatment group bled, compared to  $76.2 \pm 12.0\%$  of the sites in the placebo group; this difference was statistically significant ( $p=0.008$ ).

## **Gingival Index**

At T1, GI was  $1.56 \pm 0.14$  and  $1.68 \pm 0.12$  in the treatment and control groups respectively. The groups were significantly different ( $p=0.029$ ) (Figure 4). From T1 to T2 there was a  $0.14 \pm 0.11$  reduction in GI of the treatment group and a  $0.07 \pm 0.18$  reduction in the control group. The group difference in the reductions that occurred approached, but was not statistically significant ( $p=0.059$ ). However, the changes of the treatment group were statistically significant ( $p=0.002$ ), whereas those of the placebo group were not ( $p=0.182$ ) (Figure 5). At T2, the GI of the treatment group was  $1.42 \pm 0.17$  compared to  $1.61 \pm 0.20$  in the control group, which was a statistically significant ( $p=0.006$ ) difference. Between T2 and T3, the GI of the treatment and control groups increased  $0.13 \pm 0.15$  and  $0.13 \pm 0.20$ , respectively; the group difference was not statistically significant ( $p=0.789$ ). The changes within groups were statistically significant for both the treatment ( $p=0.010$ ) and control groups ( $p=0.048$ ). At T3, the GIs of the treatment and control groups were  $1.54 \pm 0.14$  and  $1.74 \pm 0.14$ , respectively, representing a significant ( $p=0.001$ ) group difference.

## **Probing Depth**

At T1 the PDs of the treatment and control groups were  $2.58 \pm 0.16$  mm and  $2.60 \pm 0.21$  mm, respectively, with no significant ( $p=0.663$ ) group difference (Figure 6).

From T1 to T2 the changes were statistically insignificant between the groups ( $p=0.443$ ), and within the treatment ( $p=0.570$ ) and control groups ( $p=0.379$ ). The treatment group decreased  $0.03 \pm 0.15$  mm, and the control group increased  $0.05 \pm 0.16$  mm (Figure 7).

At T2 the PD in the treatment group was  $2.54 \pm 0.09$  mm and  $2.65 \pm 0.11$  mm in the control group, which was a statistically significant ( $p=0.019$ ) difference. From T2 to T3, the treatment group increased  $0.08 \pm 0.12$  mm, and the control group decreased  $0.0004 \pm 0.105$  mm, which were not significantly ( $p=0.065$ ) different. However, the increase within the treatment group was statistically significant ( $p=0.033$ ), while the small decrease in the control group was not ( $p>0.999$ ). At the T3 follow-up visit, the difference between groups was not statistically significant ( $p=0.715$ ); the treatment and control groups had PDs of  $2.61 \pm 0.09$  mm, and  $2.65 \pm 0.13$  mm, respectively.

## **Plaque Index**

Initially, the PI was  $0.95 \pm 0.29$  in the treatment group and  $1.04 \pm 0.36$  in the control group, with no significant group difference ( $p=0.431$ ) (Figure 8). From T1 to T2 the changes were small and statistically insignificant for both the treatment ( $p=0.427$ ) and control ( $p=0.233$ ) group; the group difference in the changes that occurred was also

statistically insignificant ( $p=0.135$ ) (Figure 9). At T2, the PI in the treatment group had slightly reduced to  $0.87 \pm 0.24$  and the control group had increased to  $1.17 \pm 0.41$ , with no group difference ( $p=0.051$ ). From T2 to T3, PI in the treatment and control groups increased  $0.18 \pm 0.25$  and decreased  $0.01 \pm 0.34$ , respectively, with no statistically significant ( $p=0.234$ ) group difference. The increase within the treatment group was statistically significant ( $p=0.028$ ) while the decrease within the control group was not ( $p=0.900$ ). The PI at T3 was  $1.05 \pm 0.32$  in the treatment group and  $1.14 \pm 0.32$  in the control group, again with no group difference ( $p=0.452$ ).

## CHAPTER V

### DISCUSSION

Compared to the control group, the treatment group demonstrated decreased severity of gingivitis and inflammation. This is evidenced by a 13.6 percentage point (21.6%) reduction in BOP during the T1 to T2 treatment interval, compared to a 3.0 percentage point (4.1%) reduction in the control group. Additionally, GI decreased from 1.56 to 1.42 (8.9%), whereas the GI of the control group decreased only 4.2%, from 1.68 to 1.61. The treatment effect was further validated by the subsequent increase in BOP and GI that occurred following cessation of treatment (T2-T3). The reduction in gingivitis during treatment may have been due to decreases in inflammatory mediators such as TNF- $\alpha$  and various interleukins brought about by the antioxidant component of the gel.<sup>23, 38</sup> The essential oil component may also be a factor, since it has been shown to improve BOP and GI in in-vivo studies.<sup>11, 13, 35, 36</sup>

The treatment group did not show any clinically significant improvement in PD compared to the control group. There was a statistically significant difference between the groups at T2, but the difference was only 0.11 mm. The treatment group also showed a statistically significant increase in PD after cessation of the gel use, but the increase was only 0.08 mm. Despite the lack of clinically significant results regarding PD, this study does not preclude the possibility that this type of treatment may have a positive effect on pocket depth reductions. It must be remembered that both the treatment and control groups began the study with normal probing depths, leaving very

little room for reduction of PD. Chapple et al showed minor gains in clinical attachment levels during the initial phases of treatment with a systemic antioxidant treatment.<sup>43</sup> However, this was an adult sample with chronic periodontitis and at least two sites per quadrant having greater than 6 mm of attachment loss.

Plaque levels did not appear to be affected by treatment with the antioxidant-essential oil gel. The active treatment group showed a reduction from 0.95 to 0.87 (8.4%) from T1 to T2, while the control group increased from 1.04 to 1.17 (17%). However, the group difference was small and not statistically significant. There might not have been enough power to detect such a small effect. The lack of plaque reduction may also have been due to the plaque retentive nature of orthodontic appliances.<sup>6, 21</sup> While antioxidants have been shown to have an effect on plaque bacteria in-vitro,<sup>14, 15</sup> and essential oil mouth rinse has been shown to reduce plaque in-vivo,<sup>13, 48</sup> these effects may be nullified during orthodontic treatment.<sup>49</sup> This is consistent with findings of Tufekci et al<sup>22</sup> and Chen et al,<sup>29</sup> who showed small, and statistically insignificant, increases in PI over 6 months in orthodontic patients utilizing essential oil mouth rinses.<sup>35, 50</sup>

The reduction in gingival inflammation is clinically significant compared to the control group, but it is possible that this study underestimated the possible effect-size of treatment with an antioxidant-essential oil gel. Other studies, using the same GI criteria as this study,<sup>13, 51-54</sup> and evaluating essential-oil mouth rinses, have shown larger reductions in the GI ( $\approx 20\%$ ) than the present study (8.9%). The difference could have been due to compliance. In the present study, compliance was only evaluated verbally at

T2, with all patients responding that they used the gel twice a day as instructed, and seldom missed an application. However, estimates of compliance with homecare oral hygiene regimens have been reported to range between 68% and 82%.<sup>55</sup> It should also be noted that in self-reporting, compliance is often over-estimated.<sup>56, 57</sup> Use of a written reporting system and or periodic reminders to the patients may have increased actual compliance,<sup>58</sup> and provided a better estimate of the true effect-size in the treatment group.

The oxidative stress,<sup>9, 34</sup> cytotoxicity,<sup>8, 32</sup> and increased plaque retention<sup>6</sup> associated with orthodontic appliances may also account for the smaller effect-size in this study compared to others. Two recent studies involving essential oil rinse in orthodontic patients report conflicting results. Tufekci et al showed a small, but statistically insignificant, increase in their modified gingival index (MGI)<sup>59</sup> and bleeding index (BI)<sup>60</sup> over 6 months,<sup>35</sup> while Chen et al showed a 7% reduction in their MGI and a 66% reduction in BI.<sup>50</sup>

Further efforts also need to be made to determine the effect of the individual components of the gel (antioxidants and essential oils). It is also necessary to make direct comparisons to currently accepted treatment modalities for orthodontic patients, including essential oil mouth rinses<sup>35, 50</sup> and chlorhexidine rinses, which have been shown to decrease both PI and GI.<sup>13</sup> In the present study, because of the gel's formulation, it is impossible to determine whether the treatment effect was due to the antioxidants, the essential oils, or from a possible synergistic effect.



Findings within the literature indicate that patients with generalized gingival inflammation are at higher risk for attachment loss<sup>17</sup> and white spot lesions<sup>31</sup>. Treatment with antioxidant-essential oil gel may reduce the risk of attachment loss and white spot lesions in these high-risk patients.

## **CHAPTER VI**

### **CONCLUSIONS**

The study showed that topical antioxidant-essential oil gel is an effective means of reducing gingival inflammation in orthodontic patients. It reduced BOP and the GI approximately 22% (13.6 percentage points) and 9%, respectively, but had little or no effect on the PI and PD.

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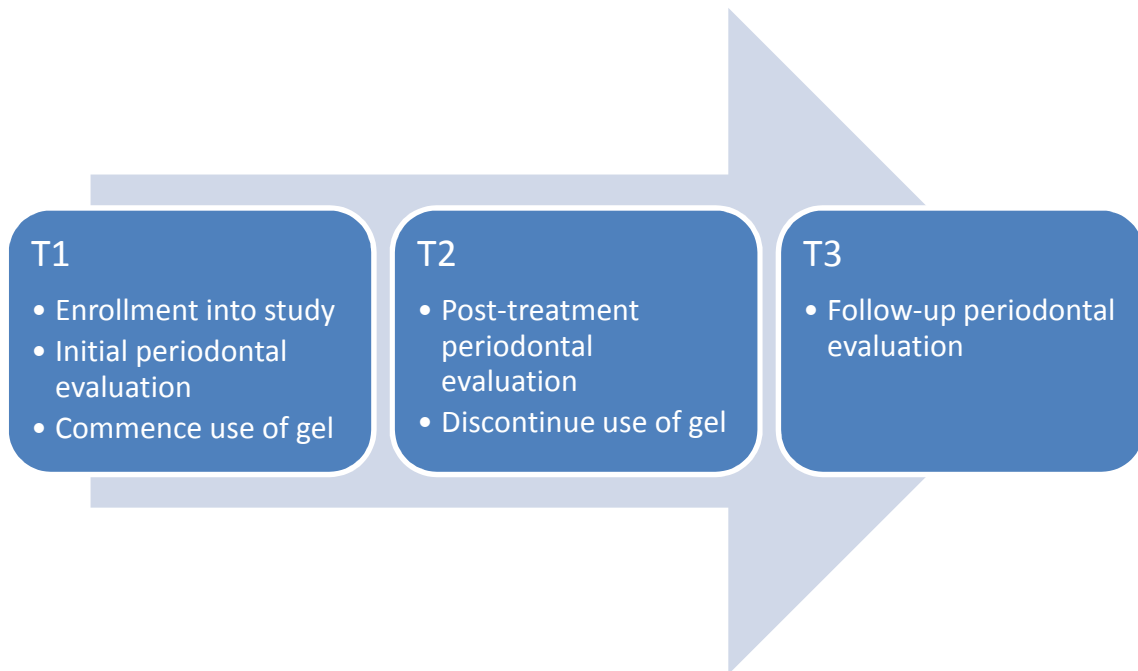
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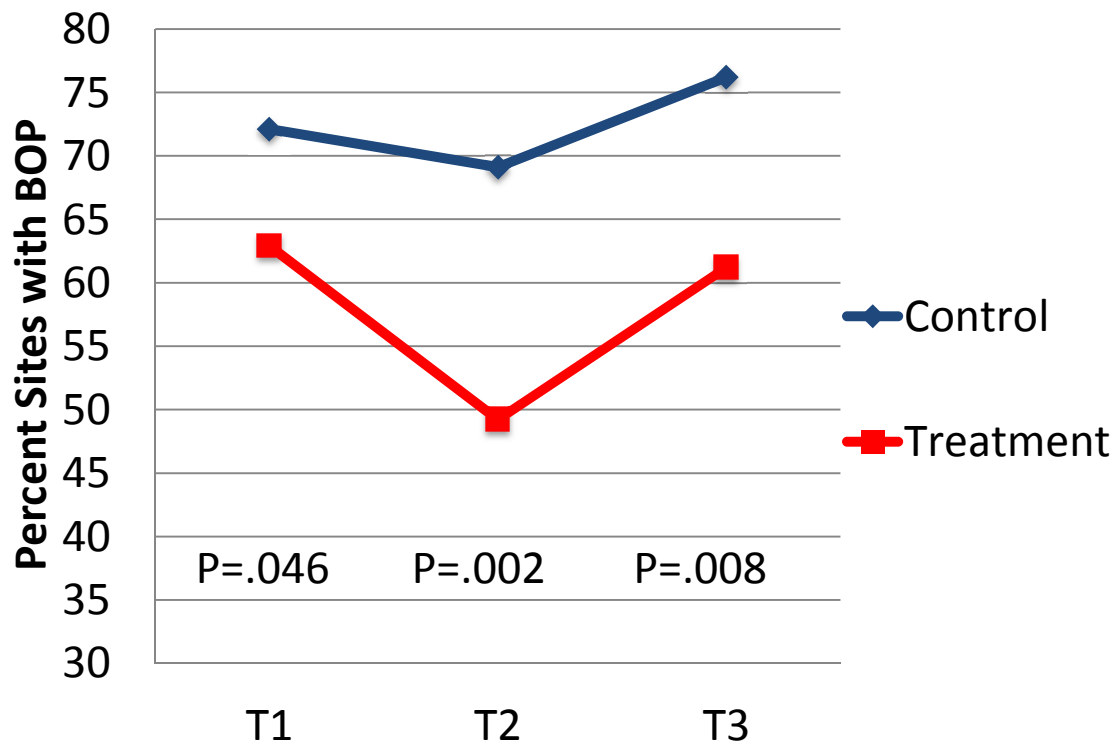
## APPENDIX A

### FIGURES

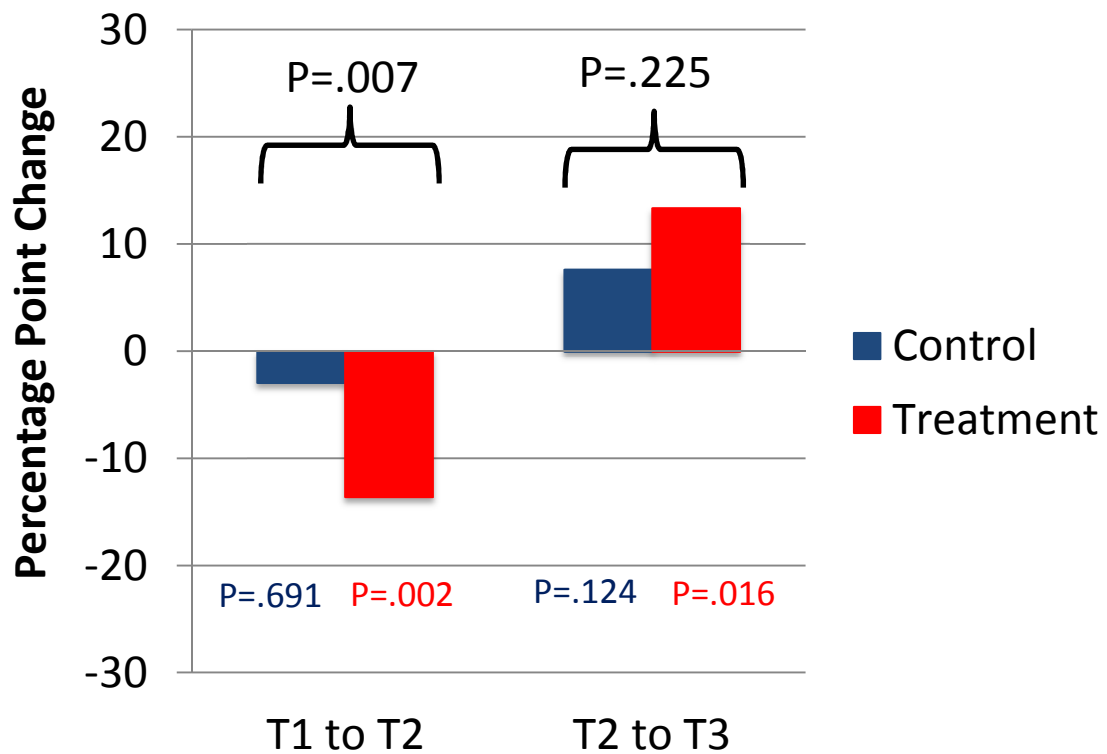


**Figure 1. Patient Flow through the Study from T1 to T3.**

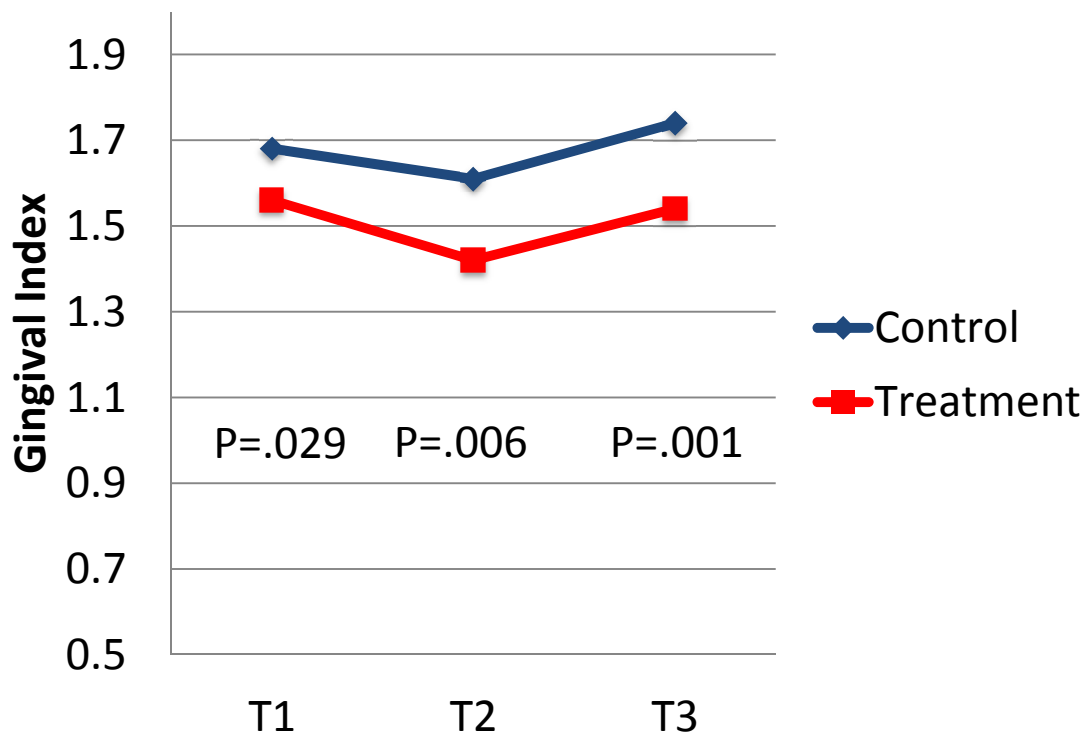




**Figure 2. Percentage of Sites with Bleeding on Probing.**  
Probability of group differences noted for each time point (Mann-Whitney U test).

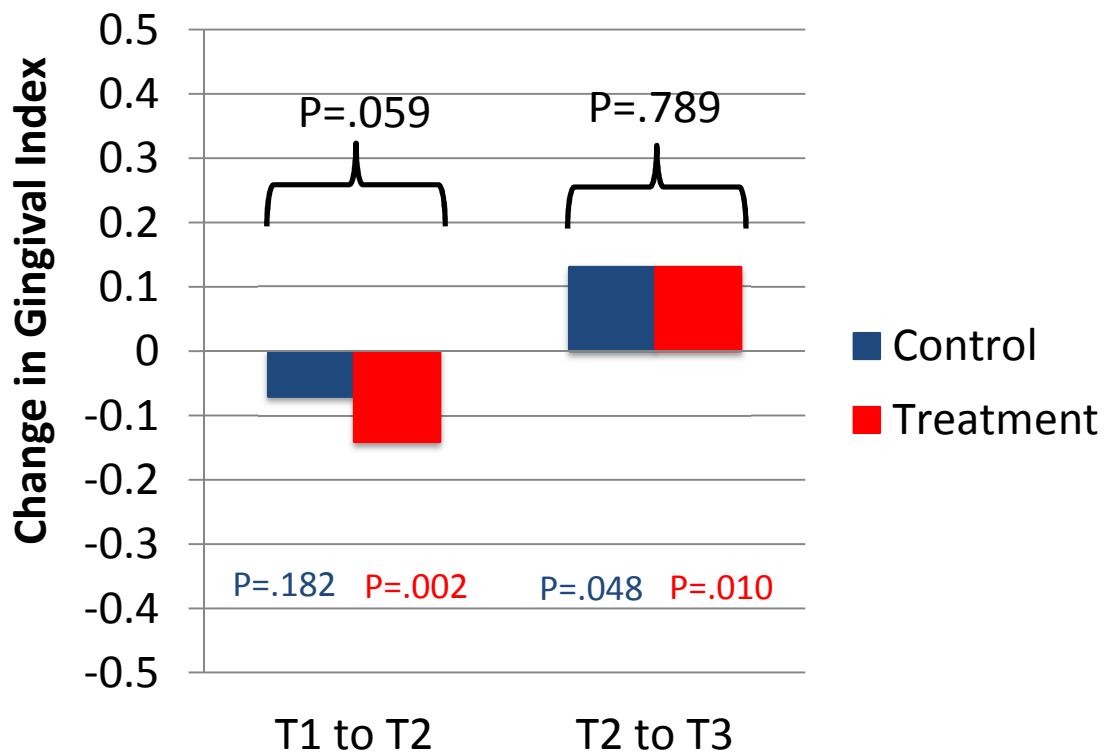


**Figure 3. Percentage Point Change in Sites with Bleeding on Probing.** Probability of group difference noted in black (Mann-Whitney U test); probability of difference between time points within a group are noted in color (Wilcoxon signed ranks test).



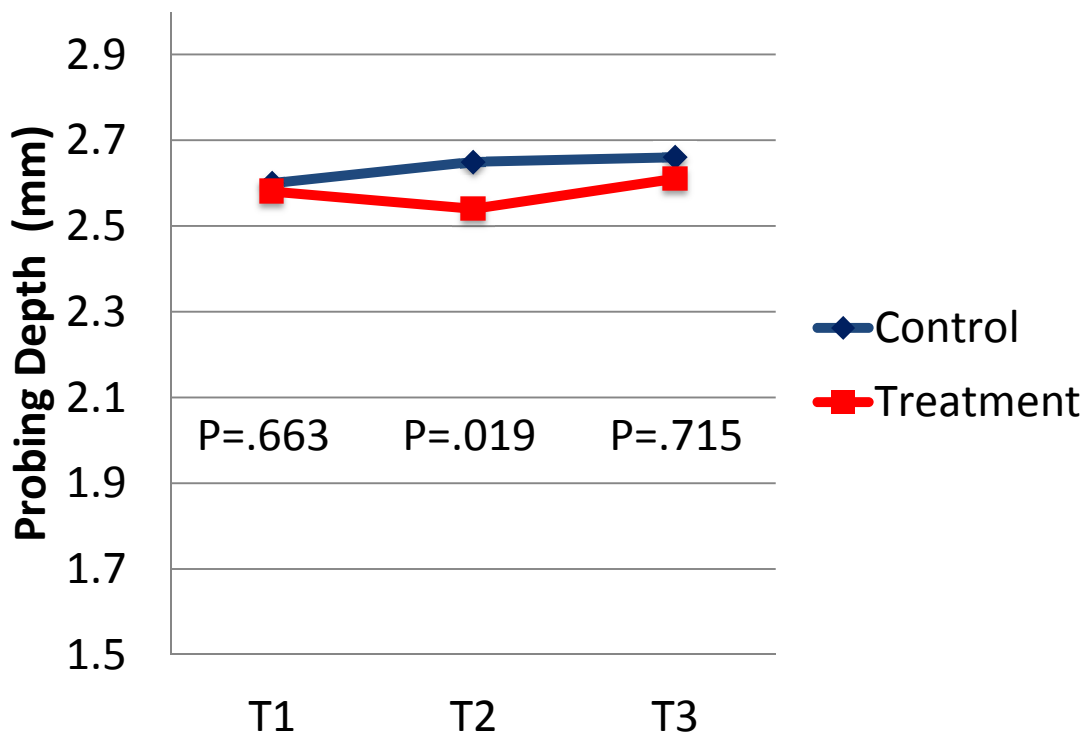
**Figure 4. Gingival Index.**

Probability of group differences noted for each time point (Mann-Whitney U test).



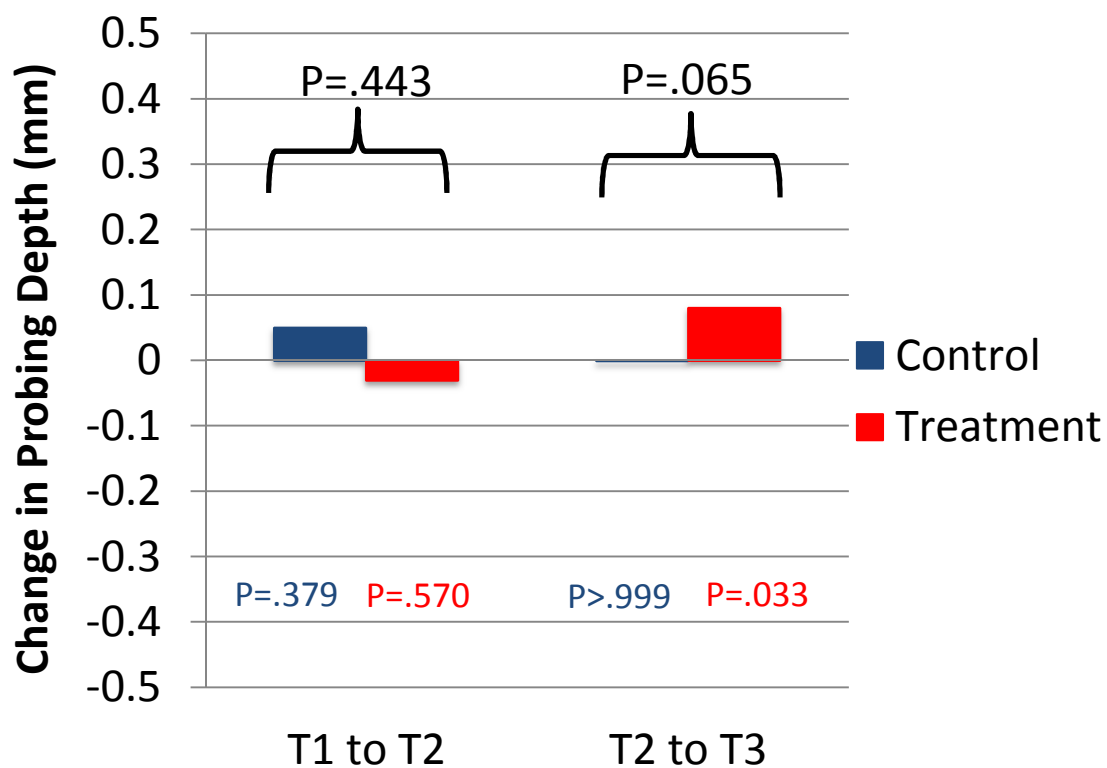
**Figure 5. Change in Gingival Index.**

Probability of group difference noted in black (Mann-Whitney U test); probability of difference between time points within a group are noted in color (Wilcoxon signed ranks test).



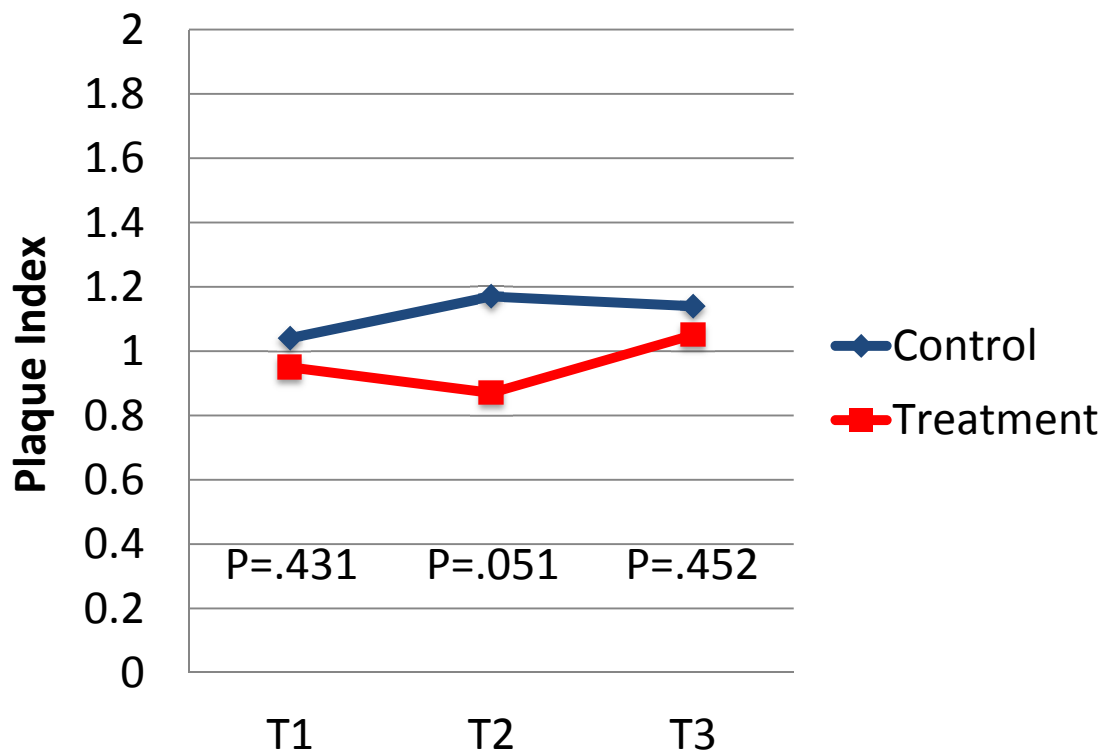
**Figure 6. Probing Depth.**

Probability of group differences noted for each time point (Mann-Whitney U test).



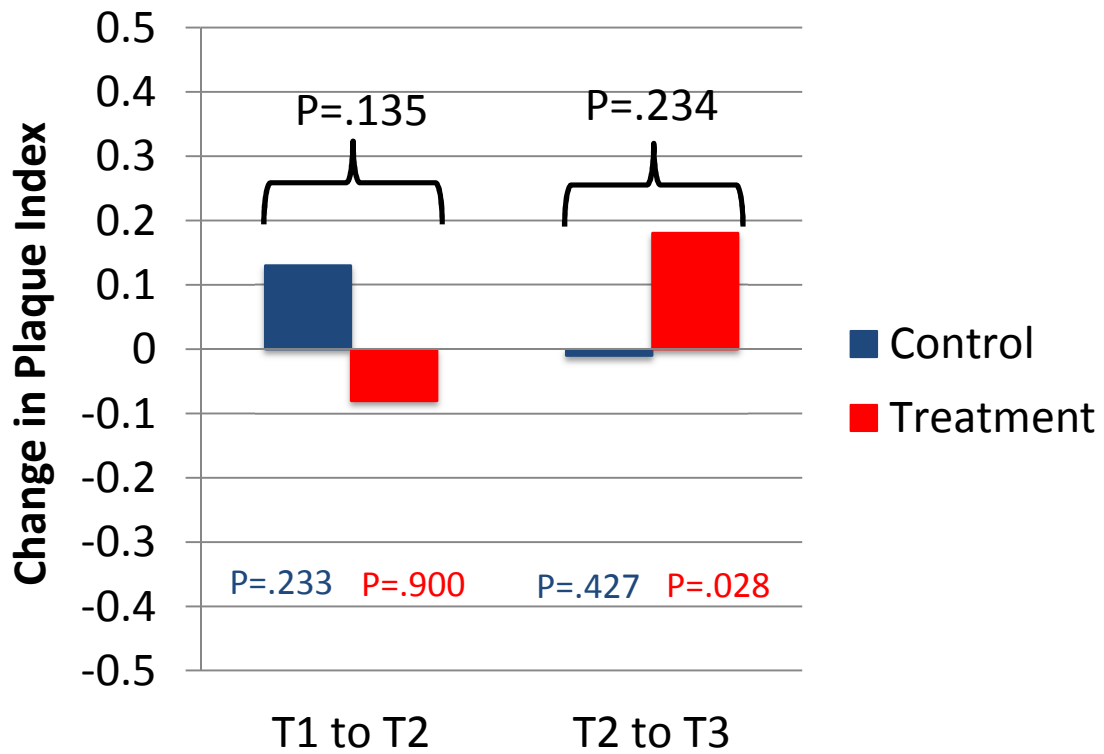
**Figure 7. Change in Probing Depth (mm).**

Probability of group difference noted in black (Mann-Whitney U test); probability of difference between time points within a group are noted in color (Wilcoxon signed ranks test).



**Figure 8. Plaque Index.**

Probability of group differences noted for each time point (Mann-Whitney U test).



**Figure 9. Change in Plaque Index.**

Probability of group difference noted in black (Mann-Whitney U test); probability of difference between time points within a group are noted in color (Wilcoxon signed ranks test).



## APPENDIX B

### TABLES

<b>% Sites with BOP</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>
<b>Treatment</b>	62.9 ± 12.9	49.2 ± 15.7	61.2 ± 9.3
<b>Control</b>	72.1 ± 10.7	69.1 ± 16.7	76.2 ± 12.0
<b>P-Value</b>	0.046	0.002	0.008
<b>Gingival Index</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>
<b>Treatment</b>	1.56 ± 0.14	1.42 ± 0.17	1.54 ± 0.14
<b>Control</b>	1.68 ± 0.12	1.61 ± 0.20	1.74 ± 0.14
<b>P-Value</b>	0.029	0.006	0.001
<b>Probing Depth (mm)</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>
<b>Treatment</b>	2.58 ± 0.16	2.54 ± 0.09	2.61 ± 0.13
<b>Control</b>	2.60 ± 0.21	2.65 ± 0.11	2.66 ± 0.13
<b>P-Value</b>	0.663	0.019	0.715
<b>Plaque Index</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>
<b>Treatment</b>	0.95 ± 0.28	0.87 ± 0.24	1.05 ± 0.32
<b>Control</b>	1.04 ± 0.36	1.17 ± 0.41	1.14 ± 0.32
<b>P-Value</b>	0.431	0.051	0.452

**Table 1. Outcome Variable Data for T1, T2, and T3.**

Probability of group differences provided (Mann-Whitney U test).

<b>Percentage point change in sites with BOP</b>	<b>T1-T2</b>	<b>T2-T3</b>	<b>T1-T3</b>
<b>Treatment</b>	-13.6 ± 10.2	+13.3 ± 14.4	-1.2 ± 8.1
<b>P-Value for changes within treatment group (Wilcoxon)</b>	0.002	0.016	0.552
<b>Control</b>	-3.0 ± 12.5	+7.6 ± 15.4	+4.1 ± 9.6
<b>P-Value for changes within control group (Wilcoxon)</b>	0.691	0.124	0.109
<b>P-Value for changes between groups (Mann-Whitney)</b>	0.007	0.225	0.139
<b>Change in Gingival Index</b>	<b>T1-T2</b>	<b>T2-T3</b>	<b>T1-T3</b>
<b>Treatment</b>	-0.14 ± 0.11	+0.13 ± 0.15	-0.02 ± 0.13
<b>P-Value for changes within treatment group (Wilcoxon)</b>	0.002	0.010	0.694
<b>Control</b>	-0.07 ± 0.18	+0.13 ± 0.20	+0.06 ± 0.12
<b>P-Value for changes within control group (Wilcoxon)</b>	0.182	0.048	0.074
<b>P-Value for changes between groups (Mann-Whitney)</b>	0.059	0.789	0.099
<b>Change in Probing Depth (mm)</b>	<b>T1-T2</b>	<b>T2-T3</b>	<b>T1-T3</b>
<b>Treatment</b>	-0.03 ± 0.15	+0.08 ± 0.12	+0.04 ± 0.14
<b>P-Value for changes within treatment group (Wilcoxon)</b>	0.570	0.033	0.279
<b>Control</b>	+0.05 ± 0.16	0.00 ± 0.10	+0.06 ± 0.15
<b>P-Value for changes within control group (Wilcoxon)</b>	0.379	> 0.999	0.363
<b>P-Value for changes between groups (Mann-Whitney)</b>	0.443	0.065	> 0.999
<b>Change in Plaque Index</b>	<b>T1-T2</b>	<b>T2-T3</b>	<b>T1-T3</b>
<b>Treatment</b>	-0.08 ± 0.24	+0.18 ± 0.25	+0.07 ± 0.31
<b>P-Value for changes within treatment group (Wilcoxon)</b>	0.427	0.028	0.463
<b>Control</b>	+0.13 ± 0.43	-0.01 ± 0.34	+0.10 ± 0.49
<b>P-Value for changes within control group (Wilcoxon)</b>	0.233	0.900	0.402
<b>P-Value for changes between group (Mann-Whitney)</b>	0.135	0.234	0.771

**Table 2. Outcome Variable Changes between Time Points.**

Probability of group differences (Mann-Whitney U test), and probability of differences within a group between time points (Wilcoxon signed ranks test) provided.